VEGETATIVE WASTE OF FOOD INDUSTRY AS BASE TO PRODUCE FUNCTIONAL FOODS, BIOADDITIVES AND BIOSORBENTS

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Abstract.

The technology for processing waste plant material for their further use as food or dietary products *"Lignin-Cellulose Sorbents"* and functional foods *"CELISORB"* was developed. It was studied the organoleptic and physicochemical parameters of these materials and was shown that they were promising materials for use in medicine and food industry as (entero)sorbents, antioxidants and dietary supplements, which could solve many problems of internal ecology of organism (endoecology) and health of people.
Integrated radiometric, laboratory and clinical research were carried out. Clinical studies were done to assess the therapeutic and decorporative efficacy relative to ^{137}Cs for children aged 7-17 residing in territories contaminated with radionuclides.

Introduction

Despite the fact that 25 years past since the Chernobyl disaster, the problem of removal of radionuclides from the human bodies remains true today and will always have over the years, especially in connection with the recent events at Japanese nuclear power plant "Fukushima-1". The leading role in protecting the people from chronic "ecological poisoning" is played by efferent medicine, first of all by sorption therapy and prophylaxis. Therefore one of priority directions of developing the modern technology is the protection of the people against toxic and harmful substances by creation of new cheap and effective adsorbents. The resource for production of such materials can be a multi-tonnage solid wastage of food-processing industry – shell of nuts, apricot, peach or plum stones, vegetable, apple, olive and grape seed, pulp, peels of grain cultures, basket and peel of sunflower, corncob, cacao husks, coffee waste, seaweeds etc. Functional foods, bioadditives and biosorbents on the basis of processing vegetative raw materials are interesting for ecology, medicine and the food-processing industry. The main structural components of such wastage are cellulose, pectin, alginates, and lignin tight in to biopolymer complexes. In contrast to synthetic ones, these substances are "soft" (according to the effect on the mucous membrane) and biologically compatible with human body.

In the progress of this the technologies of preparation of functional lignin-cellulose materials were developed [1]: it was created plant material for use in the food industry as a dietary supplement *Lignin-Cellulose Sorbents* (TU U

15.8-03291669-008:2008), and functional food product *CELISORB* (TU U 15.8-03291669-014:2010).

Experimental part

The modification of plant materials (nuts shell, grape and fruit stones, wheat and sunflower peel, etc.) is based on partial depolymerisation of macromolecules of cellulose-lignin complexes under influence of various physical and chemical factors, selective oxidizing of alcoholic groups up to carbonyl and carboxyl ones, obtain of ethers with compounds containing additional groups with acidic properties.

The surface area of the dry fiber samples was determined from nitrogen adsorption isotherms measured at 77 K by AUTOSORB (Quantachrome) computer-controlled surface analyzer.

The combined physical and chemical (NMR, IR, and SEM) studies have been carried out on the content of the compositionally heterogeneous lignin-cellulose conglomerates and the nanostructured molecular organization of lignin and its biocomplexes with cellulose has been determined.

IR-spectra of samples of native and lignin-enriched samples were obtained by reflection method. Spectrum recording was carried out by spectrophotometer M-40 in the wavelength range of 400-4000 cm^{-1}.

The componential composition of lignin-cellulose complexes in powdered samples is determined by spectra of NMR CP MAS ^{13}C by AVANCE 400 (Bruker). The spectra were registered in a pulse mode of accumulation with working frequency of 100.3 MHz and cross-polarization on frequency of 400.13 MHz (H1); the interval between impulses was 4 sec. A speed of rotation of samples under a magic corner consisted 12 kHz. The values of chemical shifts for ^{13}C atoms were defined concerning standard TMS (δ = 0 ppm).

To determinate the structural change in componential stock of lignin-cellulose complexes the electronic microphotographs of initial and powdery samples of investigated materials (SEM, with energy dispersive analyzer EDS/EDX/EDAX and ESEM, JEOL model JSM-5500LV) were obtained and analyzed.

Clinical efficacy was assessed by a special uniform protocol: "Protocol assessment of clinical efficacy of food with radioprotective properties", where the data of clinical observation, the presence of side effects and allergic reactions, the results of laboratory and instrumental studies were recorded. The content of cesium-137 in children body was controlled by metrological secured LVL SKRYNER-3M.

The blood was studied in semi-automatic analyzer "SYSMEX" (Japan), including the following: leukocyte count, red blood cells, platelets, hemoglobin concentration.

Population and subpopulation composition of immune competent cells was studied by flow cytofluoromety (FACStar Plus, "Becton Dickinson" company).

The level of serum immune globulins major classes A, M, G was estimated by the method of simple radial immune diffusion in agar gel.

The intensity of free radical processes in the biological media of children body (serum and red blood cells) was determined by initiated chemiluminescence.

It was estimated the light-sum (S) of initiated chemiluminescence (integral indicator that characterizes the rate of use of free radicals due to their interaction with antioxidants) and the amplitude (H) of fast flash (an indicator that reflects the content of hydroperoxides in the lipid substrate that is investigated).

It was determined also the condition of the digestive system, in particular, hepatobiliary system and pancreas and performance of coprograms. Using standard ultrasound sonography "Aloka SSD-500" (Japan) the structure of thyroid tissue was also studied.

Results and discussion

It should be marked that after modification the specific surface area of vegetative materials substantially changes (from 1-2 up to 10-20 m^2/g), and micro- and meso-porosity of samples arises.

The presence in processed plant materials mesoporous structure provides for the sorption of large oligo- and polymer molecules (globules) of physiologically active substances.

To determine the character of interaction of various ions with functional groups of plant complexes it was studied IR-spectra of the last.

Thus, comparative analysis of the spectra of untreated and treated materials (fig. 1) shows a slight decrease in intensity of the bands associated with a decrease in energy between the inner and molecular hydrogen bonding polymers, chemically treated. Violation of the stability of intermolecular bonds "cellulose - lignin – others polysaccharides" under the virtues of hydrolyzing chemicals leads to the extraction from plant material hemicelluloses, pectin's, fats, waxes, and partial degradation of cellulose.

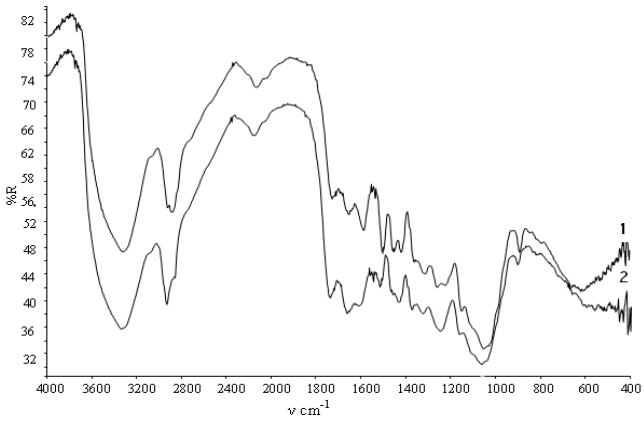


Fig. 1. IR-spectra of native (1) and modified (2) sunflower shucks.

Reducing "diffuseness" a broad absorption band in the range of 3100-3600 cm^{-1} due to stretching vibrations of hydroxyl groups included in the hydrogen bonds and can be attributed to a reduction of intermolecular interactions by hydrogen bonds of lignin and cellulose increase the availability of lignin due to removal of hydrophobic components and "loosening" structure of the plant material.

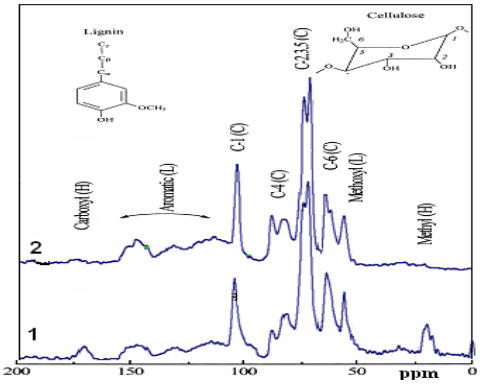
For example, the spectra of the husk, preprocessed by chemical modifiers it can be seen the changes in the range of 3000-2800 cm-1, where there are characteristic absorption bands of mono- and polyphenols, i.e. lignin. In this interval, there are absorption bands due to stretching vibrations of =CH- and $-CH_2-$ groups. Thus, the intensity of the bands at 2917 and 2852 cm^{-1} is reduced in comparison with native husk, which may be associated with changes in the structure of the substrate.

The presence in the IR spectrum of native husk absorption bands at 1646, 1664, 1740 cm^{-1} in the range of 1800-1600 cm^{-1} due to the presence in the sample of carboxyl groups. The spectrum in this range has a rather complicated structure, which is due to different nature of the carboxyl groups. The spectrum of the lignin-enriching samples the intensity of the band at 1740 cm^{-1} is markedly reduced. The intensity of the absorption in the range 1800 - 1600 cm^{-1} can affect the moisture contained in the husk.

The absorption bands at 1600-1510 cm^{-1} belong to skeletal vibrations of aromatic structures. The spectrum of native husk is characterized by adjacent bands at 1568, 1560 and 1520 cm^{-1}. On the spectra of the modified husk such configuration is expressed less intensely. In the range 1210-830 cm^{-1} in the spectra lignin-enriched husk takes place the reduce intensity of the absorption bands caused by vibrations of C-C and C-O bonds.

Thus, changes in the spectra of chemically modified materials indicate changes in the structure of the material due to the deterioration of inter- and intramolecular bonds of biopolymers by the modifying agents.

The analysis of the received ^{13}C NMR spectra (more detail see [2]) shows that the initial and modified samples of wood nut shell, corn cabbage stumps and cacao vela contain signals with the following value of δ: 20-40 ppm from $-CH_3$ and $-CH_2-$ groups of hemicelluloses; 60 ppm from methoxy-groups of lignin; 65 ppm from carbon atoms C-6; 70-75 ppm from atoms C-2,3,5 of cellulose; 80-90 ppm from atoms C-4 of cellulose; 120-160 ppm from aromatic carbon atoms of lignin; 175 ppm from carboxyl carbon atoms of hemicelluloses.



CP MAS NMR-^{13}C spectra of the modified samples (fig. 2) differ from spectra of predecessors by essential reduction of intensity of signals $-CH_3$ and $=CH_2$ groups of hemicelluloses (20-40ppm), and also alkyl (30-33 ppm) and carboxyl (170-175 ppm) groups of hemicelluloses.

Fig. 2. 13C NMR-spectra of hazelnut shell: (1) – initial; (2) – modified.

Comparing ^{13}C NMR spectra of investigated samples with literary data, it is possible to assign the signals observed in the field of 5–45 ppm to carbon atoms in groups >CH–, $–CH_2–$, $–CH_3$, non-bonded with atoms of oxygen, in lateral aliphatic chains between aromatic rings of lignin. The total number and position of signals in spectra of all the samples coincide. In the spectra of investigated lignin-cellulose complexes, there are signals with δ = 53.5 and 53.8 ppm testified to presence of coumaran and pinoresinol fragments in macromolecules. In investigated lignin-cellulose materials, there are also two accurate resonant signals of OCH_3-groups: δ = 55.7 and 55.9 ppm. It is known that a signal with δ = 55.6 ppm is caused by methoxyl group in ortho position, and a signal of 56.0 ppm presents carbon atoms in methoxyl groups of syringyl rings.

As it was already mentioned, the area of 100–160 ppm of a spectrum contains resonant signals of carbon atoms with sp^2-hybridizations of valent electrons involved in aromatic and olefin structures.

So, the results of the analysis of nuclear magnetic resonance ^{13}C CP MAS spectra on chemical shifts of resonant signals testify that lignin macromolecules, which are a part of lignin-celluloses complexes of investigated samples, are built by structural units of guaiacyl, syringyl, and n-coumarone types.

It is also seen that signals of hemicelluloses groups disappear, and intensity of other lines (groups of cellulose and lignin) in-creases. This testifies to structural changes in lignin-cellulose skeleton due to its modifying.

SEM microphotographs of the surface of initial and modified apricot stones (fig. 3) show concentric lamination of the cellular wall invoked by distinctions in chemical composition and orientation of structural elements, and confirm development of porosity of the material after carrying out of acid-alkaline processing.

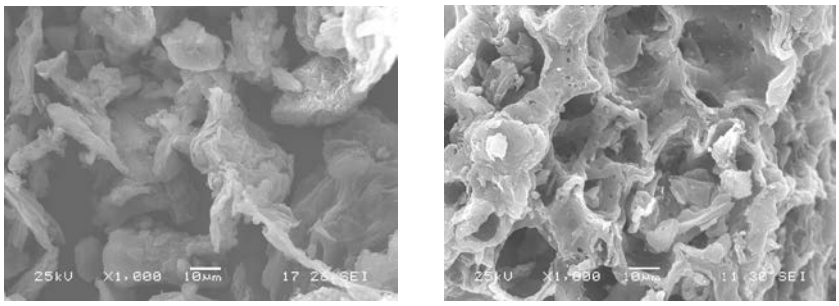


Fig. 3. SEM microphotographs of initial (left) and modified (right) apricot stones.

For example, at electron microscopy study of cacao shell the presence of assemblies of the big dimension of the wrong shape and globular fragments in initial samples was found.

Accordingly to literature data they are lignin globules composed from its macromolecules. In the samples of modified cacao shell globular assemblies are not observed. It is visible the spiral structures on a surface of plant cells. Apparently it is separate fibrils of the secondary wall of a cell (cellulose molecule).

It is also possible to see the layers of a cellular wall and sharp borders between separate layers. Among cells there is a middle plate, which does not contain the cellulose (excepting individual fibrils) and binds cells in the uniform fabric. After carried out modification there is visible the structure of elements of the cellulose named fibrils.

SEM microphotographs of a surface of initial and modified apricot stones show concentric lamination of the cellular wall invoked by distinctions in chemical composition and orientation of structural elements, and confirm development of porosity of the material after carrying out of acid-alkaline processing.

Clinical studies were carried out on the assessment of decorporative and therapeutic efficacy of lignin-cellulose preparation *CELISORB* concerning ^{137}Cs for children aged 7-17 years old - permanent residents of the territories contaminated with radionuclides.

The children of the main and control groups did not have significant differences in the activity of ^{137}Cs in the bodies. The final radiometric survey (after 20 days) showed that the activity of radiocesium in children bodies had decreased almost in twice (see Table).

Group	The content of ^{137}Cs in the body, Bq		P	Lowering, %
	Before research	After research		
main	1009±69	496±81	< 0.05	49.2
control	1278±176	993±150	> 0.05	27.0

CELISORB exhibits an immune-modulating effect, optimizing the ratio of immune-regulatory T-cell imbalance and reducing the concentration of circulated immune complexes. *CELISORB* possesses antioxidant properties. Its assignment leads to a decrease in intensity of free radical processes in the body, as indicated by normalization of initiated chemiluminescence of erythrocytes and serum (see Table).

Index	Main group		Control group	
	before	after	before	after
H red blood cells	113±10	68±7	98±13	87±19
S red blood cells	58663±6323	30356±4326	40275±9340	33611±8594
H blood serum	166±11	104±10	165±15	159.3±11
S blood serum	57844±4661	53125±5421	55374±8769	52795±7558

S – light-sum of initiated chemiluminescence; **H** – amplitude of fast flash.

An additional assignment of *CELISORB* for more than 20 days is to increase in the effectiveness of basic therapy and to normalize the level of bilirubin. Side effects are not observed.

Conclusions

The results of applied research serve as a scientific basis for environmentally sound and practically useful lingo-cellulose sorbents that will enable their practical use in solving some problems of chemical engineering, ecology, and medicine (see also [3,4]).

Food and nutrition professionals will continue to work with the food industry, allied health professionals, the government, the scientific community, and the media to ensure that the public has accurate information regarding functional foods and thus should continue to educate themselves on this emerging area of food and nutrition science.

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